Divergent Epigenetic Trajectories in Recovery and Pathology of Heat Illness

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Abstract

Introduction

Exertional heat stress causes a spectrum of illnesses, ranging from mild heat exhaustion to life-threatening heat stroke. While epigenetic mechanisms, such as DNA methylation, are thought to influence heat acclimation and recovery, the molecular changes that define the severity and progression of heat illness remain poorly understood. This study aimed to characterize and compare the longitudinal dynamics of DNA methylation in peripheral blood across the clinical spectrum of exertional heat illness.

Material and Methods

A cohort of 87 individuals diagnosed with heat exhaustion (n=32), heat injury (n=16), or heat stroke (n=39) was studied. Whole-blood DNA methylation profiles were assessed at the time of diagnosis and across four subsequent time points representing acute, sub-acute, and later recovery phases. Differential methylation analysis, time-course clustering, and pathway enrichment were used to identify epigenetic changes and their associated biological pathways.

Results

At diagnosis, heat stroke and heat injury exhibited thousands of differentially methylated probes compared to heat exhaustion, with most changes being unique to each condition. Longitudinal analysis revealed that methylation profiles in heat exhaustion normalized rapidly, indicating a quick recovery. In contrast, heat injury showed transient activation of pathways related to neuronal protection, stress regulation, and adaptive immunity, followed by sustained suppression of synaptic and T-cell signaling pathways, suggesting a resource-conserving recovery state. Heat stroke was characterized by delayed and persistent activation of maladaptive pathways, including inflammasome activation, proteotoxic stress, and oxidative strain, reflecting a progression toward systemic immune and cellular dysfunction.

Conclusions

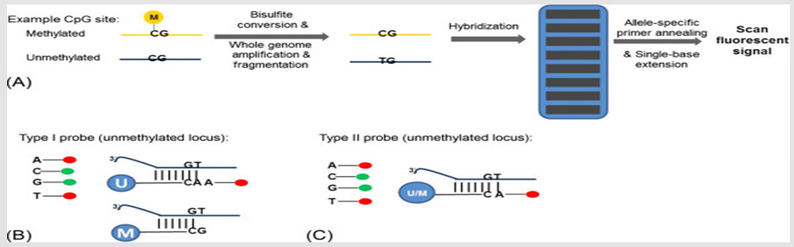
Heat illness severity is associated with distinct and persistent epigenetic trajectories in peripheral blood. While heat injury is marked by adaptive methylation programs that promote recovery, heat stroke triggers pathological signatures of systemic inflammation and cellular stress. These findings provide a molecular framework for understanding heat illness progression, identify potential biomarkers for predicting clinical outcomes, and highlight therapeutic targets to mitigate long-term complications. This study was funded by US Army Medical Research and Development Command (USAMRDC).

Methods

Cohort Description:

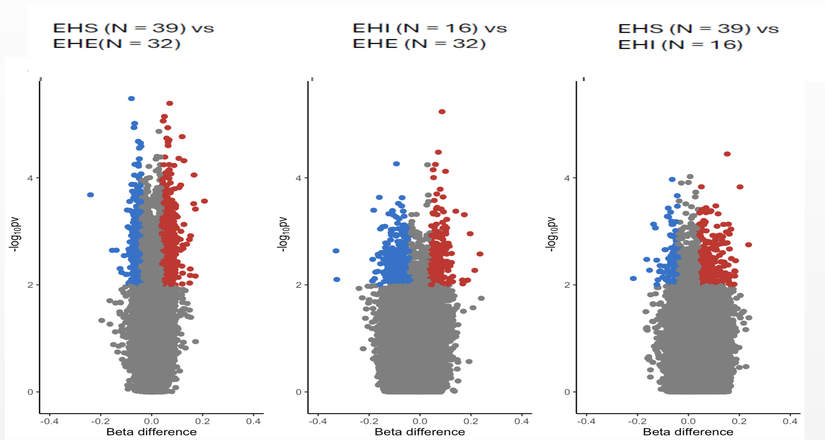
Variable	Heat Exhaustion	Heat stroke	Heat Injury
Only T=1	28	9	5
T1 = (1, 2days)	14	45	18
T2 = (3, 4 days)	3	37	6
T3 = (5, 6, 7, 8 days)	0	52	6
T4 = (>8days)	0	43	3

Assay: DNA methylation assay

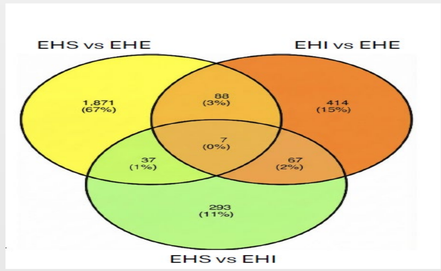


Data Analysis: DNA methylation data was analyzed for differential analysis adjusted for age, sex, race, and blood cell-type composition.

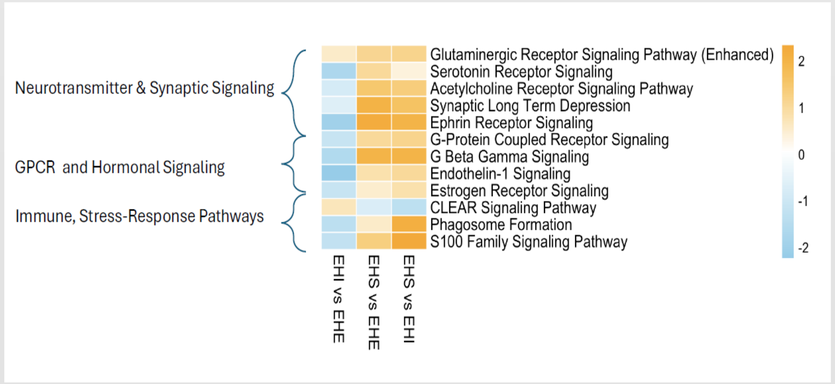
Differential Analysis



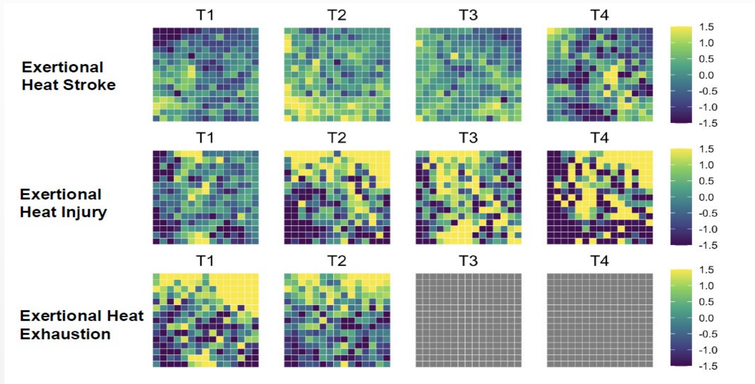
Significantly overlapping methylated regions



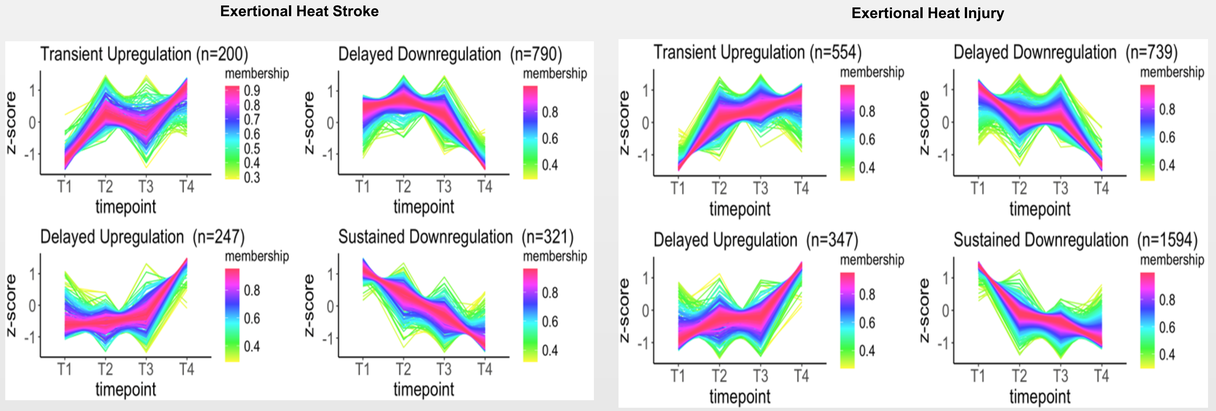
Significantly Enriched networks



global DNA methylation Patterns



Temporal Clustering of DNA methylation Patterns



Future Directions

- We identified DNA methylation signatures with each of heat stress related outcomes (exertional heat stress stroke/injury/exhaustion).
- The methylation analysis was assessed for longitudinal timepoints where collection days were grouped into four broader time bins.
- Follow-up studies should focus on -
  - Whole-blood methylome analyses represent aggregate signals across cell types and do not capture tissue-specific events.
  - Site specific validation may be required.

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